High-density lipoprotein-mediated anti-atherosclerotic and endothelial-protective effects: a potential novel therapeutic target in cardiovascular disease

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Abstract: Reduced levels of high-density lipoprotein cholesterol (HDL) are associated with a substantially increased risk of coronary disease and cardiovascular events. Furthermore, numerous studies have suggested that HDL may exert several potentially important antiatherosclerotic and endothelial-protective effects. In particular, the promotion of reverse cholesterol transport, i.e. cholesterol efflux from lipid-loaded macrophages in atherosclerotic lesions and the subsequent cholesterol transport back to the liver, has been proposed as an anti-atherogenic effect of HDL that may promote regression of atherosclerotic lesions. Moreover, endothelial dysfunction is thought to play a critical role in development and progression of atherosclerosis and several recent studies have suggested that HDL exerts direct endothelial-protective effects, such as stimulation of endothelial production of the anti-atherogenic molecule nitric oxide, anti-oxidant, anti-inflammatory and anti-thrombotic effects. Furthermore, it has been observed that HDL may stimulate endothelial repair processes, involving mobilisation and promotion of endothelial repair capacity of endothelial progenitor cells. The relative significance of these different potential anti-atherosclerotic effects of HDL remains still unclear at present. Importantly, at the same time it has been recognized that the vascular effects of HDL may be variable, i.e. the capacity of HDL to stimulate macrophage cholesterol efflux and endothelial-protective effects may be altered in patients with inflammatory or cardiovascular disease. The further characterisation of underlying mechanisms and the identification of the clinical relevance of this "HDL dysfunction" are currently an active field of research. HDL-targeted treatment strategies are at present intensely evaluated and may lead to increased HDL plasma levels and/or HDL-stimulated anti-atherosclerotic effects. The cardiovascular protection provided by such approaches may likely depend on HDL function or quality, i.e. the anti-atherosclerotic and endothelial-protective properties of the on-treatment HDL. Currently, several HDL-raising treatment strategies are examined in clinical trials, i.e. extended-release niacin, the CETP inhibitors dalcetrapib and anacetrapib, reconstituted forms of HDL (i.e. CSL-111) or apoA-I mimetics, and some of these are already in large clinical outcome studies on top of statin therapy to determine their efficacy and safety for cardiovascular prevention.

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High density lipoprotein-mediated anti-atherosclerotic and endothelial-protective effects: a potential novel therapeutic target in cardiovascular disease

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List of abbreviations

ABCA1 / G1	ATP binding cassette transporter A1 / G1
apoA-1 / B / E	Apolipoprotein A-1 / B / E
CETP	Cholesteryl ester transfer protein
eNOS	endothelial nitric oxide synthase
EPC	Endothelial progenitor cell
Flk-1	Fetal liver kinase-1
HDL	High density lipoprotein
LCAT	Lecithin-cholesterol acyltransferase
LDL	Low density lipoprotein
MAP kinase	Mitogen-activated protein kinase
MCP-1	Monocyte chemotactic protein-1
NADPH oxidase	Nicotinamide adenine dinucleotide phosphate-oxidase
NF-κB	Nuclear factor-κB
NO	Nitric oxide
PAF-AH	Platelet-activating factor acetylhydrolase
PI3-kinase	Phosphoinositide 3-kinase
PON-1	Paraoxonase-1
RCT	Reverse cholesterol transport
$S1P_{1/3}$	Sphingosine-1-phosphate receptor 1/3
Sca-1	Stem cell antigen-1
TNF-α	Tumor necrosis factor-α
VCAM-1	Vascular cell adhesion molecule-1
VLDL	Very low density lipoprotein

Abstract

Reduced levels of high density lipoprotein cholesterol (HDL) are associated with a substantially increased risk of coronary disease and cardiovascular events. Furthermore, numerous studies have suggested that HDL may exert several potentially important anti-atherosclerotic and endothelial-protective effects. In particular, the promotion of reverse cholesterol transport, i.e. cholesterol efflux from lipid-loaded macrophages in atherosclerotic lesions and the subsequent cholesterol transport back to the liver, has been proposed as an anti-atherogenic effect of HDL that may promote regression of atherosclerotic lesions. Moreover, endothelial dysfunction is thought to play a critical role in development and progression of atherosclerosis and several recent studies have suggested that HDL exerts direct endothelial-protective effects, such as stimulation of endothelial production of the anti-atherogenic molecule nitric oxide, anti-oxidant, anti-inflammatory and anti-thrombotic effects. Furthermore, it has been observed that HDL may stimulate endothelial repair processes, involving mobilisation and promotion of endothelial repair capacity of endothelial progenitor cells. The relative significance of these different potential anti-atherosclerotic effects of HDL remains still unclear at present. Importantly, at the same time it has been recognized that the vascular effects of HDL may be variable, i.e. the capacity of HDL to stimulate macrophage cholesterol efflux and endothelial-protective effects may be altered in patients with inflammatory or cardiovascular disease. The further characterisation of underlying mechanisms and the identification of the clinical relevance of this "HDL dysfunction" are currently an active field of research. HDL-targeted treatment strategies are at present intensely evaluated and may lead to increased HDL plasma levels and/or HDL-stimulated anti-atherosclerotic effects. The cardiovascular protection provided by such approaches may likely depend on HDL function or quality, i.e. the anti-atherosclerotic and endothelial-protective properties of the on-treatment HDL. Currently, several HDL-raising

treatment strategies are examined in clinical trials, i.e. extended-release niacin, the CETP inhibitors dalcetrapib and anacetrapib, reconstituted forms of HDL (i.e. CSL-111) or apoA-I mimetics, and some of these are already in large clinical outcome studies on top of statin therapy to determine their efficacy and safety for cardiovascular prevention.

Keywords: High density lipoprotein; Endothelium; Nitric oxide; Inflammation; Endothelial progenitor cells; Atherosclerosis; Coronary artery disease

1. INTRODUCTION

Reduced plasma levels of high density lipoprotein (HDL) cholesterol are associated with an increased risk of coronary disease [1-5], and numerous studies have suggested that HDL may exert anti-atherogenic effects, such as promoting cholesterol-efflux from lipid-loaded macrophages, anti-inflammatory and endothelial-protective effects. Accordingly, interventions to increase HDL-levels and/or HDL-function are currently intensely evaluated as a potential novel therapeutic strategy to reduce cardiovascular risk.

In the present review, we will therefore discuss potential mechanisms whereby HDL may exert atheroprotective effects and address the heterogeneity of HDL particles and function in inflammatory and cardiovascular disease. Importantly, recent data suggest that the vascular effects of HDL can be markedly altered in patients with diabetes or inflammatory diseases. Finally, we will provide an overview of potential therapeutic strategies increasing HDL plasma concentrations and/or function.

1.1 HDL cholesterol plasma levels, genetic variants leading to altered HDL cholesterol plasma levels and coronary heart disease risk

Numerous studies have indicated that lower plasma levels of HDL cholesterol are associated with an increased risk of coronary disease and coronary disease-related cardiovascular events [1-4]. Furthermore, it has been observed, that this does also apply to patients with coronary disease and very low levels of low density lipoprotein (LDL) cholesterol on statin therapy, as suggested by a post-hoc analysis of the Treating to New Targets trial [5]. More recently, a large analysis involving more than 300,000 people without initial vascular disease, mostly from Europe and North America, demonstrated that reduced HDL cholesterol levels were strongly associated with an increased coronary heart disease risk, whereas no such association was observed for triglyceride levels after adjustment for other cardiovascular risk factors [6].

Several recent studies have examined the association of genetic variations leading to altered HDL plasma levels, i.e. due to ATP binding cassette transporter A1 (ABCA1) mutations or genetic variants of the cholesteryl ester transfer protein (CETP), with coronary disease risk [7, 8]. In a study from Denmark, reduced HDL cholesterol levels were associated with an increased risk of ischemic heart disease, however, lower plasma levels of HDL cholesterol due to heterozygosity for four rare loss-of-function mutations in the ABCA1 gene were not associated with an increased risk of ischemic heart disease in this study [7]. The number of subjects being heterozygous for the rare ABCA1 mutations in this analysis was, however, rather low, i.e. 109 subjects, that may limit conclusions about clinical outcome in these subjects. Furthermore, subjects with the rare ABCA1 mutations had also 25% lower LDL cholesterol levels, that may potentially offset a risk associated with the reduced HDL levels [9]. In a study examining three genotypes for CETP, that were associated with a moderate inhibition of CETP activity and, therefore, modestly higher HDL cholesterol levels, a weakly inverse association with a reduced coronary risk was observed [8].

2. POTENTIAL ANTI-ATHEROGENIC AND ENDOTHELIAL-PROTECTIVE EFFECTS OF HDL

In recent years, several functions of HDL have been identified, that may account for the ability of HDL to protect against atherosclerosis (Fig. **1**) [10-12]. Besides promoting macrophage cholesterol efflux and reverse cholesterol transport, HDL has been shown more recently to exert direct vasoprotective effects, such as endothelial-protective, anti-inflammatory and anti-thrombotic effects, that will be discussed in detail below. Although the basis of atheroprotection provided by HDL is complex and has yet to be fully clarified, recent evidence suggests that the vascular effects of HDL may be highly heterogenous and vasoprotective properties of HDL may be limited in certain patient populations [13, 14].

2.1 HDL and reverse cholesterol transport from macrophages

The term macrophage reverse cholesterol transport (RCT) is generally used to describe the process by which unesterified cholesterol is exported from macrophages in atherosclerotic lesions of the arterial wall and returned to the liver for excretion in the bile [15].

2.1.1 Macrophage cholesterol efflux

The first step of macrophage RCT includes the hydrolysis of cytoplasmatic cholesterol esters to free cholesterol and the efflux of free cholesterol to extracellular lipid-poor apolipoprotein (apo) A-I or mature HDL. Early on, efflux of cholesterol from macrophages was thought to be primarily mediated by passive diffusion [16]. Now it has become clear that macrophage cholesterol efflux is facilitated by active tranport systems, including the ABCA1, ATP binding cassette transporter G1 (ABCG1) and scavenger receptor class B type I (SR-BI) [15, 17, 18].

Studies in macrophages from ABCA1 knockout or overexpressing mice have indicated that ABCA-1 primarily mediates cholesterol efflux to lipid poor apoA-I [19-21]. Of note, transplantation of bone marrow from ABCA1 knockout mice into hypercholesterolemic apoE- or LDL receptor-deficient mice resulted in an increase in atherosclerotic lesion progression, suggesting that cholesterol efflux via ABCA1 indeed plays an important role in preventing atherosclerotic vascular disease [22]. However, rates of cholesterol excretion into the bile were normal in ABCA1 knockout mice [23] indicating that the quantitative role of ABCA1 in contributing to macrophage cholesterol efflux and RCT *in vivo* remains to be further characterized. Moreover, these studies could not prove that the increased atherosclerosis in ABCA1 knockout mice was due to impaired macrophage cholesterol

efflux, but could also have other explanations, such as ABCA1-dependent export of oxidized phospholipids from vascular cells [15].

In contrast to ABCA1, ABCG1 and SR-BI largely mediate cholesterol efflux from macrophages to mature HDL, which might be more efficient in mediating RCT *in vivo* than lipid poor apoA-I, also since mature HDL represents a much larger proportion of HDL and apoA-I found in plasma as compared to the limited amount of lipid-poor apoA-I [24]. However, studies characterizing the effect of ABCG1 and SR-BI on macrophage RCT and development of atherosclerosis in mice have yielded controversial results. Whereas ABCG1-deficient mice displayed increased diet-induced lipid accumulation in macrophages [25] and one group reported accelerated early atherosclerotic lesion development in these mice [26], other groups found a decreased atherosclerotic plaque burden in atherosclerosis prone mice transplanted with ABCG1-deficient bone marrow [27, 28]. Potential explanations for these conflicting results might be a compensatory upregulation of ABCA1 and apoE, increased apoptosis of macrophages due to lipid accumulation and the stage of atherosclerotic lesion development [29]. Of note, a recent study quantitatively assessed the roles of ABCA1, ABCG1 and SR-BI in macrophage RCT *in vivo* [30]. By using primary macrophages lacking SR-BI the authors were able to demonstrate that SR-BI did not promote macrophage RCT *in vivo* after intraperitoneal injection in wild type mice [30]. In contrast to SR-BI, ABCA1 and ABCG1 contributed in an additive fashion to stimulation of macrophage RCT *in vivo* [30] and transplantation of bone marrow from ABCA1 / ABCG1-deficient mice accelerated atherosclerotic lesion formation in LDL receptor-deficient mice [31]. Consistent with these findings, Yvan-Charvet et al. [32] were able to demonstrate that SR-BI fails to stimulate net cholesterol efflux from HEK293 cells to plasma HDL and inhibits ABCG1-mediated cholesterol efflux, at least in part due to an increased uptake of HDL cholesteryl esters into HEK293 cells. These studies suggest that previous findings of accelerated atherosclerosis in

apoE- and LDL receptor-deficient mice transplanted with bone marrow from SR-BI deficient mice [33, 34] cannot be explained by decreased macrophage cholesterol efflux.

2.2.2 Cholesterol esterification by lecithin-cholesterol acyltransferase (LCAT)

Following efflux from macrophages, a proportion of HDL-associated free cholesterol in plasma is esterified to cholesteryl ester by lecithin-cholesterol acyltransferase (LCAT). Although LCAT activity allows the formation of a hydrophic core in HDL and thereby plays an important role in the formation of mature HDL particles, the relevance of LCAT for RCT remains controversial [35]. Recently, it has been suggested that LCAT activity is not required for the ability of human plasma to promote macrophage cholesterol efflux *in vitro* [36] and may have a minor role for macrophage RCT *in vivo* [37], consistent with the observation that HDL directly transfers a large amount of unesterified cholesterol to the liver for biliary cholesterol excretion [38, 39]. Moreover, a recent study suggested that carotid intima media thickness was not increased in a cohort (n=40) of individuals with loss-of-function mutations in LCAT [40].

2.2.3 Cholesterol uptake to the liver

The final step of RCT comprises the uptake of HDL-associated cholesterol to the liver. HDL cholesterol can be delivered directly to the liver by selective uptake of cholesteryl ester and unesterified cholesterol by hepatic SR-BI [15, 41]. Of note, overexpression of hepatic SR-BI increased macrophage RCT in mice, although plasma levels of HDL cholesterol were reduced [42], suggesting that hepatic SR-BI expression is inversely related to atherosclerosis development. Alternatively, in humans and in some other species HDL cholesterol can be transferred to apoB-containing lipoproteins in exchange for triglycerides via the cholesteryl ester transfer protein (CETP) and subsequently be cleared by LDL receptor-mediated uptake

of apoB-containing lipoproteins to the liver. Indeed, in mice with CETP gene transfer (that normally lack CETP) there was evidence for an increased macrophage RCT [43]. However, HDL isolated from human subjects with homozygous CETP deficiency had an increased cholesterol efflux potential [44]. Therefore, the relevance and effect of modulation of CETP activity or levels for macrophage cholesterol efflux and RCT remains to be further determined.

2.2 HDL and stimulation of endothelial NO-synthase dependent nitric oxide (NO) production

In recent years, numerous experimental and clinical studies have suggested that endothelial production of nitric oxide (NO) plays a crucial role in the regulation of vascular tone and structure [45, 46]. Besides stimulation of endothelium-dependent vasodilation, endothelial NO has been shown to exert a variety of atheroprotective effects in the vasculature, such as anti-inflammatory, anti-thrombotic, anti-coagulant and pro-fibrinolytic effects [45]. Therefore, endothelial NO-synthase (eNOS) derived NO is considered as a critical determinant of vascular homeostasis and an important anti-atherogenic molecule. Endothelial dysfunction, likely a consequence of reduced endothelial NO bioavailability, is considered to play an important role in the development and progression of atherosclerosis [45].

The understanding of the vascular effects of HDL considerably changed with the important observation that HDL may directly stimulate eNOS-mediated NO production and induce endothelium-dependent, NO-mediated vasodilation [47]. Meanwhile, several experimental studies have consistently demonstrated the capacity of HDL to modify eNOS expression and activity and to stimulate endothelial production of NO *in vitro* and *in vivo* [14, 48-51]. Moreover, in humans, administration of reconstituted HDL has been shown to correct impaired endothelium-dependent, NO-mediated vasorelaxation in subjects with isolated low

levels of HDL due to a ABCA1 mutation as well as in patients with hypercholesterolemia by restoring NO bioavilability [52, 53].

Several different mechanisms have been proposed to account for the endothelial NO-stimulating capacity of HDL (Fig. **2**). Early studies have suggested that HDL acts by preventing the detrimental effects of oxidized LDL on endothelial NO-synthase [54]. A subsequent study by Yuhanna et al. [47] suggested that HDL can directly stimulate eNOS-mediated NO production by binding to SR-BI on endothelial cells.

Although the signaling events underlying stimulation of eNOS upon binding of HDL to SR-BI are complex, recent studies have provided important insights into proximal mechanisms that are involved in HDL-induced signal transduction in endothelial cells (Fig. **2**). Binding of HDL to SR-BI initially leads to tyrosine kinase Src-mediated activation of phosphoinositide (PI) 3-kinase and PI3-kinase in turn activates Akt and the MAP kinase/extracellular signal-regulated kinase pathway [49]. Activation of endothelial Akt by HDL has been shown to stimulate phosphorylation of eNOS at serine residue 1177 [49, 51], that is known to be an important regulatory mechanism leading to eNOS activation [55]. In contrast, the mechanism whereby the MAP kinase/extracellular signal-regulated kinase pathway activates eNOS in endothelial cells stimulated with HDL remains to be further characterized [56].

However, in these studies isolated apoA-I, i.e. the major SR-BI ligand of HDL, failed to activate eNOS, suggesting that other HDL components besides apoA-I are likely to be important for the eNOS-stimulating capacity of HDL. Interestingly, stimulation of eNOS-mediated NO production is also induced by binding of HDL-associated lysophospholipids (i.e. sphingosylphosphorylcholine, sphingosine-1-phosphate, lysosulfatide) to the lysophospholipid receptor $S1P_3$ (Fig. **2**), that is expressed in endothelial cells and may partially mediate HDL- and lysophospholipid-induced vasodilation [51, 56]. Of note, the vasodilatory response to HDL was not completely inhibited in $S1P_3$ deficient mice. This may

suggest an interaction between SR-BI and $S1P_3$ to induce HDL signalling in endothelial cells. An interaction of HDL with SR-BI could provide the necessary spatial proximity for lysophospholipids to effectively stimulate $S1P_3$ [51]. However, further studies are needed to characterize the most proximal signalling events occurring upon binding of HDL to endothelial cells in more detail.

Recently, Terasaka et al. [57] identified another mechanism whereby HDL may maintain endothelial cell NO availability in mice fed a high-cholesterol diet. These authors suggested that ABCG1-mediated efflux of oxysterols from endothelial cells may represent a novel effect whereby HDL promotes preservation of endothelial NO production (Fig. **2**). In particular, 7-ketosterol, a dietary oxysterol, accumulated in endothelial cells of ABCG1 deficient mice on a western diet *in vivo* [57]. Interestingly, incubation of human aortic endothelial cells with HDL prevented 7-ketosterol-induced production of reactive oxygen species and disruption of the active eNOS dimer, suggesting that the ability of HDL to preserve endothelial function in the presence of hypercholesterolemia may at least in part relate to an increased endothelial efflux of 7-oxysterols [57].

2.3 HDL and anti-oxidant effects

Accumulation and subsequent oxidation of LDL in the subendothelial space is thought to play an important role in the initiation and progression of atherosclerotic vascular disease, by leading to a pro-atherogenic form of LDL, i.e. oxidized LDL, that is taken up by scavenger receptors of macrophages [58]. In recent years, several studies have demonstrated that oxidatively modified LDL promotes endothelial cell inflammatory activation [59]. Modification of LDL by 1-electron oxidants, such as tyrosyl radical or nitrogen dioxide radical, leads to the formation of lipid hydroperoxides and advanced products of oxidation, i.e. alkanes, aldehydes and isoprostanes [60-62]. Of note, HDL has been identified as a

major carrier of both early and late products of lipid oxidation [63, 64]. Moreover, it has been suggested that apoA-I, the main protein constituent of HDL, is capable of binding and removing lipid hydroperoxides of LDL *in vitro*, after injection into mice and after infusion in humans *in vivo* [61, 62]. HDL contains several anti-oxidant enzymes that may be involved in degradation of lipid hydroperoxides, such as paraoxonase-1 (PON-1), platelet-activating factor acetylhydrolase (PAF-AH), LCAT and reduced glutathione selenoperoxidase [65]. In particular, PON-1 has been suggested to be an important regulator of the anti-atherogenic capacity of HDL [66, 67]. Mice deficient in PON-1 displayed significantly larger aortic atherosclerotic lesions than their wild type controls and HDL isolated from mice deficient in PON-1 was unable to prevent oxidation of LDL in a cell co-culture model of the arterial wall [66].

However, others have questioned the hypothesis that HDL directly acquires lipid hydroperoxides from LDL or is oxidized in plasma, because the transfer of lipid hydroperoxides between LDL and HDL appears to be slow and the plasma contains several anti-oxidant defense mechanisms [60]. Another explanation for the enrichment of lipid hydroperoxides in HDL could be the fact that HDL binds lipid oxidation products, such as 7-ketocholesterol [57, 68], at sites of inflammation and transports them back to the plasma, thereby protecting endothelial cells against inflammatory activation [60]. Accordingly, Nicholls et al. [69] have reported that reconstituted HDL inhibits superoxide production and vascular inflammation induced by a non-occlusive carotid periarterial collar in normocholesterolemic rabbits. In addition, Van Linthout et al. [70] have recently observed that human apoA-I gene transfer in rats with streptozotocin-induced diabetes mellitus resulted in a 1.9-fold increase in HDL cholesterol levels and reduced diabetes-induced angiotensin II type 1 receptor expression in the aorta of diabetic rats. Concomitantly, the increased NAD(P)H oxidase activity observed in diabetic rats was inhibited by human apoA-I gene

transfer and this was at least in part due to a reduced mRNA expression of NOX4 and p22phox, two NAD(P)H oxidase subunits [70]. These data indicate that at least under hyperglycaemic conditions HDL may also exert anti-oxidative effects in the vascular wall by directly inhibiting angiotensin II type 1 receptor-mediated NAD(P)H oxidase activation and generation of reactive oxygen species.

2.4 HDL and vascular and cardiac anti-inflammatory effects

Atherosclerosis is considered as a chronic inflammatory disease. The endothelial adhesion and subsequent infiltration and accumulation of monocytes/macrophages and T lymphocytes into the arterial intima represents a critical step in the initiation and progression of atherosclerotic lesions [58]. Notably, several studies have suggested that HDL can exert a variety of anti-inflammatory effects in endothelial cells, such as inhibition of the expression of monocyte chemoattractant protein (MCP)-1, an important pro-inflammatory chemokine that acts as an attractant for inflammatory cells adherent to endothelial cells [71, 72]. Likewise, several studies have demonstrated that native HDL and reconstituted HDL containing apoA-I or the apoA-I_{Milano} mutant inhibits the expression of leukocyte adhesion molecules in endothelial cells that are activated by pro-inflammatory stimuli [73-76]. In addition, native HDL has been suggested to inhibit endothelial monocyte adhesion induced by oxidized LDL [77] or TNF-α [76] and monocyte transmigration in co-cultures of HAEC and smooth muscle cells stimulated with LDL [71]. *In vivo*, infusions of reconstituted human HDL reduced VCAM-1 expression and decreased monocyte/macrophage infiltration following carotid artery cuff injury in apoE deficient mice [78]. Interestingly, in a recent study it was shown that apoA-I gene transfer did not only increase HDL cholesterol plasma levels, but also inhibited diabetes-induced myocardial mRNA expression of VCAM-1 and ICAM-1 in mice with streptozotocin-induced diabetic cardiomyopathy [79].

In contrast, in apoE deficient mice with transgenic overexpression of human apoA-I, endothelial VCAM-1 expression was not reduced in the aortic branch sites and was not associated with reduced monocyte adherence, despite reducing aortic atherosclerotic lesion formation [80]. Hence, the anti-inflammatory capacity of HDL may be heterogeneous. This is in line with findings of recent studies demonstrating that the inhibitory effects of HDL isolated from different human subjects on TNF-α stimulated endothelial VCAM-1 expression varied considerably [81, 82]. The reasons for the heterogeneous effects of HDL on endothelial cell inflammatory activation will have to be further defined. Interestingly, more recently, it has been suggested that administration of reconstituted HDL (supplied by CSL Behring AG) increased the anti-inflammatory capacity of HDL from patients with type-2 diabetes [83].

In this regard, *in vitro* studies using discoidal reconstituted HDL containing apoA-I as the sole protein suggested that the inhibitory effects of HDL on endothelial cell adhesion molecule expression are likely, at least in part, dependent on the HDL-associated phospholipid species [84]. The inhibition of cytokine-induced expression of VCAM-1 in human umbilical vein endothelial cells (HUVEC) by reconstituted HDL varied substantially when different phosphatidylcholine species were compared, supporting the concept that the composition of phospholipids in HDL may play a role for the anti-inflammatory capacity of HDL [12, 84].

Several mechanisms have been proposed to account for the inhibitory effect of HDL on endothelial inflammatory activation [12]. The ability of HDL to stimulate endothelial NO availability and to reduce superoxide production in endothelial cells may contribute to the anti-inflammatory effects of HDL, although direct evidence for this has not yet been published. Both, impaired endothelial NO bioavailability and increased endothelial superoxide production have been implicated in activation of the important pro-inflammatory

transcription factor NF-κB [85] and independent observations have demonstrated that HDL inhibits NF-κB activity [75, 76]. Interestingly, inhibition of TNF-α stimulated NF-κB activity by HDL was, at least in part, related to a decreased activity of endothelial sphingosine kinase [75]. Sphingosine kinase catalyzes the conversion of sphingosine to sphingosine-1-phosphate in endothelial cells, that represents an important step in the pathway by which TNF-α stimulates expression of adhesion molecules in the endothelium.

2.5 HDL and inhibition of endothelial apoptosis and stimulation of endothelial repair

Functional or structural disruption of endothelial integrity in response to major cardiovascular risk factors or by direct mechanical injury (i.e. after percutaneous coronary intervention), induces a variety of pro-inflammatory and proliferative responses in the arterial wall [86]. These responses likely contribute to initiation and progression of atherosclerotic plaque formation, vascular remodelling and development of restenosis [87]. Therefore, there is increasing interest in novel therapeutic approaches that maintain endothelial integrity by preventing endothelial cell apoptosis or promoting endothelial repair [86].

Of note, HDL has been shown to inhibit apoptosis of endothelial cells induced by several stimuli, such as TNF-α [88], oxidized LDL [89] and growth factor deprivation [90], i.e. HDL may likely inhibit death-receptor-mediated and mitochondrial-mediated apoptotic pathways (Fig. **3**). Depending on the pro-apoptotic stimulus, different compounds of HDL have been suggested to mediate the anti-apoptotic capacity of the lipoprotein. ApoA-I has been implicated in inhibition of endothelial cell apoptosis induced by oxidized LDL [89], VLDL [91] and TNF-α [88]. Interestingly, a recent study suggested that HDL subpopulations enriched with apoA-I account for approximately 70% of the anti-apoptotic activity of HDL in human microvascular endothelial cells that were treated with mildly oxidized LDL and reconstitution of HDL with apoA-I, cholesterol and phospholipids potently decreased

oxidized LDL-induced apoptosis in these cells [92], suggesting that apoA-I indeed plays an important role for the anti-apoptotic capacity of HDL in oxidized LDL-stimulated endothelial cells.

In contrast, HDL-associated lysosphingolipids have been shown to inhibit endothelial cell apoptosis induced by growth factor depletion [90, 93, 94]. The anti-apoptotic effects of sphingosylphosphorylcholine and lysosulfatide, two bioactive lysosphingolipids present in HDL particles, has been suggested to blunt activation of the mitochondrial-mediated pathway of apoptosis and may lead to activation of Akt that is known to be an important kinase for anti-apoptotic signalling in endothelial cells [90]. The role of HDL-associated lysosphingolipids for the anti-apoptotic effects of HDL was further suggested by another study demonstrating that the ratio of sphingosine-1-phosphate and sphingomyelin was increased in small dense HDL3 particles and positively correlated with the capacity of HDL subpopulations to attenuate endothelial cell apoptosis [95].

More recent work has also suggested that HDL may stimulate endothelial repair processes (Fig. **3**). Whereas endothelial repair process have long been thought of to be only dependent on the proliferation and migration of local adjacent endothelial cells [96], several recent studies have clearly suggested that bone-marrow-derived endothelial progenitor cells (EPC) may promote endothelial repair after vascular injury [97-99], contribute to endothelial repair processes in lesion-prone areas of experimental atherosclerosis [100] and improve endothelial function [101].

Recent work has suggested that HDL stimulates endothelial repair both, by promotion of endothelial cell proliferation or migration and stimulation of the recruitment and endothelial repair capacity of EPC [14, 102, 103]. HDL has consistently been shown to induce a marked increase in endothelial cell migration *in vitro* that is comparable to the effect of endothelial growth factors, such as basic fibroblast growth factor or vascular endothelial growth factor

[94, 102, 104]. Kimura et al. [94] demonstrated that native HDL and the HDL-associated lysosphingolipid sphingosine-1-phosphate stimulate migration of HUVEC and migration induced by HDL was potently inhibited either by antisense oligonucleotides against the sphingosine-1-phosphate receptors $S1P_1$ and $S1P_3$ or pertussis toxin (i.e. by inhibiting interactions between G proteins and G protein-coupled receptors). Moreover, the sphingosine-1-phosphate-rich fraction of HDL and sphingosine-1-phosphate purified from HDL stimulated endothelial cell migration, whereas the fraction containing other bioactive lysosphingolipids did not, suggesting that HDL-induced endothelial cell migration may be mediated by binding of sphingosine-1-phosphate to $S1P_1$ and $S1P_3$ [94]. The importance of sphingosine-1-phosphate for endothelial cell migration was supported by another study demonstrating that sphingosine-1-phosphate induced tube formation of human coronary artery endothelial cells *in vitro* by Ras/Raf1-dependent ERK activation [105].

In contrast, in a work by Seetharam et al. [102] pertussis toxin did not affect HDL-mediated endothelial cell migration, suggesting the presence of another pathway and agonist, which induces the migration of endothelial cells by HDL. Indeed, the authors observed that reconstituted HDL consisting of apoA-I, palmitoyloleylphosphatidylcholine and cholesterol was able to induce endothelial cell migration [102]. Moreover, native HDL induced rapid changes in the actin cytoskeleton of endothelial cells (i.e. a decrease in stress fibers, an increase in lamellipodia, and membrane ruffling) paralleled by an activation of the small GTPase Rac, that is known to mediate lamellipodia formation [102]. Interestingly, the authors were able to demonstrate that endothelial Rac activation and migration in response to HDL is independent of endothelial NO production, but requires binding of HDL to SR-BI and activation of Src kinase, PI3-kinase and MAP kinase [102]. Recently, the same group identified PDZ domain-containing protein PDZK1 as an adaptor protein of SR-BI in endothelial cells and suggested that PDZK1 is required for the initiation of HDL signalling

via SR-BI in endothelial cells and plays an important role for endothelial cell migration induced by HDL [103].

Interestingly, further studies have suggested that HDL and SR-BI promote re-endothelialization of the carotid artery after perivascular electric injury in mice [14, 102]. In this model, carotid artery re-endothelialization was impaired in apoA-I deficient mice with low HDL levels as well as in SR-BI deficient mice [102]. Of note, reconstitution of apoA-I expression by liver-directed apoA-I gene transfer with subsequent normalisation of HDL plasma levels restored the re-endothelialization response in apoA-I deficient mice, strongly suggesting that apoA-I and HDL promote endothelial monolayer integrity *in vivo* [102]. In another study, elevation of HDL levels in apoE-deficient mice induced by adenoviral human apoA-I (AdA-I) transfer increased the number of Flk1 / Sca-1 double-positive cells in peripheral blood and the number of DiI-acLDL / FITC-isolectin double positive cells after 4 days of *ex vivo* culture of spleen mononuclear cells [106]. Besides increasing the number of EPC, AdA-I transfer in apoE-deficient mice improved the migratory capacity of spleen-derived early EPC in response to HDL, the adhesion of spleen-derived early EPC to fibronectin and the invasion of spleen-derived early EPC in solidified Matrigel [106]. Finally, AdA-I transfer also promoted the incorporation of EPCs in Balb/c common carotid artery allografts transplanted paratopically in C57BL/6 ApoE-/- mice that was associated with an increase in endothelial regeneration and inhibition of transplant arteriosclerosis [106]. In a follow-up study, the same group observed that murine and human early EPC express SR-BI, as indicated by immunocytochemistry analysis of human early EPCs and murine spleen-derived early EPCs after 4 and 7 days of *ex vivo* culture [107]. Of note, the authors did not observe an increase in circulating Flk1 / Sca-1 double-positive cells and DiI-acLDL / FITC-isolectin double positive cells after *ex vivo* culture of spleen mononuclear cells after AdA-I transfer in mice transplanted with SR-BI deficient bone marrow, suggesting that expression

of SR-BI in bone marrow is critical for EPC mobilisation induced by HDL [107]. Furthermore, the migratory capacity of bone-marrow derived early EPC deficient in SR-BI in response to HDL was reduced as compared to early EPC containing SR-BI and this was at least in part due to an impaired activation of extracellular signal-regulated kinases (ERK) and decreased NO production in SR-BI deficient early EPC [107]. *In vivo*, SR-BI deficiency in bone marrow abrogated the inhibitory effect of AdA-I transfer on allograft vasculopathy after paratopical transplantation of a common carotid artery of a female BALB/c donor mouse into the recipient male C57BL/6 mice, that was paralleled by impaired endothelial regeneration and EPC incorporation in allografts [107].

Besides increasing HDL levels by adenoviral human apoA-I transfer, intravenous infusion of reconstituted HDL has also been shown to increase the number of Sca-1 positive cells in the aortic endothelium of apoE deficient mice, supporting a role for HDL in promoting progenitor-mediated endothelial repair [108]. Furthermore, intravenous injection of reconstituted HDL increased blood flow recovery and capillary density in a murine ischemic hindlimb model [109].

3. HETEROGENEITY OF HDL PARTICLES

Accumulating evidence suggests that the effects of HDL on cholesterol efflux from macrophages and on vascular cells, in particular endothelial cells, can be highly heterogeneous [110]. This may likely be secondary to changes in the HDL-associated proteom and lipids, i.e. changes in the amount and type of proteins and lipids bound as well as by protein and lipid modifications. In particular, oxidative modifications of HDL-associated proteins, such as apoA-I, have been observed to impair the capacity of HDL to stimulate macrophage cholesterol efflux and to exert anti-inflammatory effect [111-114].

Moreover, HDL consists of a number of discrete subpopulations that vary in size, density, and composition of lipids and apolipoproteins [115]. Of note, the capacity of HDL particles of different size or structure to exert anti-atherogenic effects may differ [110]. For the anti-inflammatory capacity of HDL it has been demonstrated that the smaller and denser HDL3 subfraction was superior to the larger and less dense HDL2 subfraction in inhibiting TNFα-induced VCAM-1 expression in endothelial cells [81]. To test whether the morphology and composition of HDL particles affects their anti-inflammatory capacity, discoidal and spherical reconstituted HDL particles of defined size and chemical composition have been studied for their ability to inhibit TNFα-induced VCAM-1 expression in endothelial cells [116]. In these studies, the anti-inflammatory capacity of HDL was not affected by variations in HDL particle size or in the composition of HDL-associated apolipoproteins, cholesteryl esters or triglycerides [116]. However, the anti-inflammatory capacity of HDL may be affected by the HDL-associated phospholipid content, as indicated by studies with reconstituted HDL containing apoA-I as the sole protein and different phosphatidylcholine species [84].

Importantly, in a seminal study by van Lenten et al. [82] it has been demonstrated that the anti-inflammatory capacity of HDL is affected by acute phase responses in both humans and rabbits. The authors isolated human HDL from the same subjects before and after cardiac surgery and characterized the effects of HDL on LDL-induced monocyte transmigration and lipid hydroperoxide formation [82]. Before cardiac surgery, HDL completely inhibited the LDL-induced increase in monocyte transmigration and lipid hydroperoxide formation. In marked contrast, “acute phase” HDL obtained from the same patients 2-3 days after surgery amplified the LDL-induced monocyte transmigration and was less effective in inhibiting lipid hydroperoxide formation, i.e. HDL in the same patient had been transformed from an anti-inflammatory towards a pro-inflammatory particle [82]. Interestingly, the changes in HDL

functionality in this study were paralled by an increase in HDL-associated acute phase reactants (i.e. coeruloplasmin and serum amyloid A), while the activities of the HDL-associated anti-oxidant enzymes paraoxonase and platelet-factor activating acetylhydrolase were reduced in acute phase HDL [82].

In subsequent years, we and others have observed that HDL loses important anti-atherosclerotic and anti-inflammatory properties in patients with chronic inflammatory disorders, such as the antiphospholipid syndrome [13], systemic lupus erythematosus and rheumatoid arthritis [117], scleroderma [118], the metabolic syndrome [119], diabetes [14, 120] and coronary disease [121]. Notably, in a study of 189 patients with chronic kidney disease on hemodialysis an impaired anti-inflammatory capacity of HDL was correlated with a poor clinical outcome, i.e. a reduced survival [122]. A further understanding of the mechanisms leading to altered vascular effects of HDL will be crucial also within the context of HDL-raising therapies, since it is likely, that only raising of HDL with vasoprotective properties will exert cardiovascular protection.

4. POTENTIAL TREATMENT STRATEGIES TO RAISE HDL CHOLESTEROL PLASMA LEVELS AND/OR IMPROVE HDL FUNCTION

The clinical potential and underlying mechanisms of HDL-targeted treatment strategies will be discussed in more detail in another review article within this issue of Curr Pharm Design [“High-density lipoprotein-raising strategies: update 2010”. Frank Spillmann, Sophie Van Linthout, Heinz-Peter Schultheiss, Carsten Tschöpe]. We will therefore largely limit the discussion here to the treatment-induced qualitative and functional changes of HDL.

4.1 Extended release niacin

More than fifty years ago, Altschul and coworkers described for the first time the effect of supraphysiological doses of nicotinic acid, that is a water-soluble vitamin of the vitamin B complex, on total cholesterol plasma levels [123]. In particular the ability of nicotinic acid to substantially increase plasma concentration of HDL cholesterol has in recent years led to a substantial interest in the pharmacological potential of nicotinic acid for cardiovascular prevention [124]. In the Coronary Drug Project, treatment with nicotinic acid (3g/d) reduced the risk of definite nonfatal recurrent myocardial infarction in patients with a previous myocardial infarction in the follow-up after 8 years [125]. In the fifteen years follow-up study a lower all-cause mortality was observed in the group that was initially randomised to nicotinic acid therapy, however, the randomised treatment duration was limited to the first 8 years [126]. Because of unfavourable side effects (i.e. cutaneous flushing and gastrointestinal side effects), the use of nicotinic acid in primary and secondary prevention of coronary disease has been limited, in particular in Europe, although nicotinic acid potently raises HDL cholesterol levels by up to 20 to 25% [127-129].

Interestingly, several recent studies have suggested that extended release (ER)-niacin therapy improves endothelial function in subjects without cardiovascular risk factors and low HDL levels and potentially in patients with coronary disease and low HDL levels [48, 130, 131]. Notably, in a recent study, we have observed that ER-niacin therapy improved the capacity of HDL from patients with diabetes to stimulate endothelial NO production, to reduce endothelial superoxide levels and to promote early EPC-mediated endothelial repair processes [14]. Furthermore, the improvement of endothelial-protective properties of HDL, i.e. HDL functionality, from type-2 diabetic patients with metabolic syndrome and low HDL levels by ER-niacin treatment were accompanied by a reduction in the activity and content of HDL-associated myeloperoxidase, an enzyme that targets tyrosine and methionine residues of

apoA-I for oxidation [132], and a lowered lipid peroxidation state of HDL in these patients [14].

Furthermore, in the HDL-Atherosclerosis Treatment Study (HATS), a combination therapy with nicotinic acid and simvastatin led to a reduction in the occurrence of a first cardiovascular event and in coronary atheroma volume in patients with coronary disease, low HDL and normal LDL cholesterol levels [133]. However, this study had a rather limited patient number to assess the effects of clinical outcome. Moreover, there was no statin monotherapy group for comparison and the low dose of simvastatin (mean = 13 mg per day) was not in line with current recommendations. Notably, in the Arterial Biology for the Investigation of the Treatment Effects of Reducing Cholesterol (ARBITER 2) study, ER-niacin has been shown to reduce the progression of carotid intima media thickening over time in patients with coronary disease on statin therapy and low HDL levels after one year of follow-up, but the effect between the ER-niacin-treated group and the placebo group did not reach statistical significance [134]. However, the follow-on open label uncontrolled crossover study, ARBITER 3, reported a regression of carotid intima–media thickness after a further 12 months of ER-niacin treatment [135]. In line with these findings, ER-niacin (2g daily) added to statin therapy significantly reduced carotid wall area as compared to placebo in a preliminary analysis of a double-blind randomized placebo-controlled study in patients with low HDL cholesterol (<40 mg/dl) and either type 2 diabetes with coronary heart disease or carotid/peripheral atherosclerosis [136]. More recently, the results of the ARBITER 6–HALTS trial (Arterial Biology for the Investigation of the Treatment Effects of Reducing Cholesterol 6–HDL and LDL Treatment Strategies) were presented addressing the question whether the addition of ezetimibe or the addition of ER-niacin is more effective in decreasing the progression of carotid intima–media thickness in patients with coronary heart disease or a coronary heart disease risk equivalent, who were receiving long-term statin therapy, and had

relatively low LDL and HDL cholesterol levels (<55 mg/dl for women or <50 mg/dl for men) [137]. In this study, the addition of extended-release niacin to statin therapy led to a decrease in the common carotid intima–media thickness, whereas no significant net changes in the carotid intima–media thickness were seen with the addition of ezetimibe [137]. In both, the HATS and the ARBITER studies the effect of ER-niacin therapy on HDL function has not been characterized.

Currently, two large outcome trials, i.e. the National Heart, Lung, and Blood Institute (NIH) co-sponsored study AIM-HIGH and the HPS2-THRIVE, examine the effects of ER-niacin therapy on cardiovascular events in high-risk patients. The AIM-HIGH (Niacin Plus Statin to Prevent Vascular Events) trial randomizes more than 3000 patients with established vascular disease, atherogenic dyslipidemia including low HDL plasma levels (men <40 mg/dl, women < 50 mg/dl) to either simvastatin or an extended-release combination therapy of niacin and simvastatin. The HPS2-THRIVE (Treatment of HDL to Reduce the Incidence of Vascular Events) trial recruits 20,000 patients in the United Kingdom, China, and Scandinavia to determine if combining niacin with a new drug (MK-0524A) that minimizes flushing can reduce cardiovascular disease risk in patients that have already reached LDL cholesterol levels < 100 mg/dl.

4.2 CETP inhibition

The cholesteryl ester transfer protein (CETP) mediates the transfer of cholesteryl esters from HDL to triglyceride-rich lipoproteins, which are subsequently cleared by hepatic LDL receptors [138]. By transferring cholesteryl esters from HDL toward apoB-containing lipoproteins *in exchange* for triglycerides, CETP decreases the concentration of HDL cholesterol and apoA-I, and increases the concentration of cholesteryl esters in VLDL and

remnants. In addition, CETP activity raises levels of LDL cholesterol and apoB, at least in part due to a downregulation of hepatic LDL receptors, as described previously [139]. Studies using neutralizing CETP monoclonal antibodies have demonstrated that CETP is responsible for all cholesteryl ester and triglyceride transfer activity in human plasma and inhibition of CETP leads to substantially increased HDL levels and a reduced cholesteryl ester content in VLDL [139].

Whereas studies in rabbits and humans have suggested that CETP does not stimulate the overall process of RCT [18, 140], a recent study in mice with CETP gene transfer suggested that CETP expression may promote macrophage RCT via the LDL-cholesterol receptor [43]. Indeed, the lipoproteins mediating RCT may differ in the presence or absence of CETP activity, i.e. in the presence of CETP activity RCT may be mediated, at least in part, by remnants and LDL, and in the absence of CETP activity HDL-dependent RCT may be more important. Notably, the direct capacity of HDL to stimulate cholesterol efflux from macrophages (ABCG1-dependent) has been observed to be rather enhanced in CETP-deficient subjects [44].

Of note, studies where the human CETP gene was introduced in atherosclerosis prone mice have yielded mixed results. In hypercholesterolemic apoE and LDL-receptor-deficient mice, and in a mouse model of mixed hyperlipidemia expressing apoE (Leiden), expression of CETP accelerated atherosclerosis [141-143]. However, in hypertriglyceridemic apolipoprotein CIII-transgenic mice expression of human CETP has been shown to be either non- or anti-atherogenic [144].

The relevance of CETP for HDL plasma levels in humans was identified first in Japanese families with inherited deficiency of CETP due to a gene splicing defect [145, 146]. Family members that were homozygous had moderate hypercholesterolemia, markedly increased levels of HDL cholesterol and apoA-I, and decreased levels of LDL cholesterol and apoB, as

compared to unaffected family members [146]. Members heterozygous for the deficiency had moderately increased levels of HDL cholesterol and apoA-I [146]. Despite these effects on the plasma lipoprotein profile, studies assessing the risk of coronary artery disease in subjects that are heterozygous for the CETP gene defect have yielded controversial results. In a cross-sectional study of 3,469 men of Japanese ancestry in the Honolulu Heart Program, CETP gene mutations leading to decreased CETP activity and increased HDL cholesterol levels were associated with an increased risk for coronary heart disease [147]. In a seven-year follow-up study in 2,340 elderly men of the same population, aged 71 to 93 years, CETP mutations were associated with a lower incidence of coronary heart disease, however, the reduction in the incidence of coronary heart disease did not reach statistical significance [148]. A recent meta-analysis on individual patient data of 13,677 subjects from 7 large, population-based studies (each >500 individuals) and 3 randomized placebo-controlled pravastatin trials suggested that the CETP *TaqIB* variant is associated with HDL-cholesterol plasma levels and more importantly with coronary disease risk [149]. Furthermore, a recent study characterizing the association of CETP genotypes leading to a moderate reduction in CETP activity and coronary risk pointed towards a weakly inverse association with coronary risk [8].

The finding of elevated HDL cholesterol levels in Japanese families with genetic CETP deficiency, spurred the interest in pharmacological inhibition of CETP as a novel therapeutic approach to raise HDL cholesterol levels. However, torcetrapib, the first CETP inhibitor that was characterized in large clinical trials, failed to reduce progression of carotid atherosclerosis in patients with familial hypercholesterolemia that were on statin treatment, and was associated with progression of disease in the common carotid segment [150]. Of note, these effects occurred despite a pronounced increase in HDL cholesterol levels and a substantial decrease in LDL cholesterol and triglycerides levels [150]. More importantly, in

the Investigation of Lipid Level Management to Understand its Impact in Atherosclerotic Events (ILLUMINATE) study, a large clinical outcome trial characterizing the effects of torcetrapib on major cardiovascular events in patients at high cardiovascular risk who were receiving statins, torcetrapib therapy was associated with an increased morbidity and mortality [151]. The exact reasons for the adverse outcome are still not entirely clear. However, in the patient population that was studied in ILLUMINATE, torcetrapib therapy was associated with a number of potential off-target effects, such as increases in blood pressure, sodium, bicarbonate and aldosterone levels, and a decrease in potassium levels, that could account, at least in part, for the increase in adverse cardiovascular events.

Importantly, two different CETP inhibitors, the smaller molecules dalcetrapib and anacetrapib, do not seem to increase blood pressure are currently evaluated in clinical trial programs [152]. Anacetrapib (MK-0859, Merck) has been shown to effectively raise HDL cholesterol levels in patients with dyslipidemia after 4 weeks of therapy without affecting blood pressure levels in a 24-h ambulatory blood pressure study [153]. Comparable effects were seen in 589 patients with hypercholesterolemia or mixed hyperlipidemia and in this study 8 weeks of anacetrapib treatment led to an increase in HDL cholesterol by up to 139% [154]. Of note, co-administration of anacetrapib with atorvastatin resulted in significant incremental LDL cholesterol reductions and similar HDL cholesterol increases, as compared to atorvastatin therapy alone [154]. An ongoing phase III trial is characterizing the effects of anacetrapib treatment on the plasma lipoprotein profile and safety in patients with coronary disease or a coronary disease risk-equivalent.

The CETP-inhibitor dalcetrapib (Roche) has been shown to confer a more modest inhibition of CETP activity and is currently evaluated in a large clinical trial program (dalcetrapib HDL evaluation, atherosclerosis and reverse cholesterol transport [dal-HEART] program), that will assess the effects of dalcetrapib on endothelial function (dal-Vessel study), atherosclerotic

disease progression (dal-PLAQUE) and cardiovascular outcome in stable patients with a recent acute coronary syndrome (dal-OUTCOME morbidity and mortality study).

Interestingly, studies from the group of Alan Tall have recently suggested that in CETP-deficient subjects as compared to normolipidemic controls and in healthy, moderately hyperlipidemic subjects after treatment with the CETP inhibitor torcetrapib the capacity of HDL to promote macrophage cholesterol efflux was rather increased [44, 155]. Similarly, Catalono et al. [156] have observed, that torcetrapib rather enhanced the subnormal capacity of HDL2 particles from dyslipidemic patients to mediate free cholesterol efflux via both SR-BI and ABCG1 pathways in hyperlipidemic subjects. However, the effect of CETP inhibition on the direct vasoprotective properties of HDL remains to be characterized.

4.3 Reconstituted HDL, apoA-I mimetics and apoA-I inducer

Experimental studies in mice using transgenic or somatic gene transfer approaches have suggested that overexpression of the human apoA-I gene leads to increases in apoA-I and HDL cholesterol [157-159], and may thereby reduce progression of atherosclerosis [157, 158]. Moreover, apoA-I overexpression in mice has been shown to promote RCT from macrophages to feces in mice [160]. Therefore, therapeutic strategies that aim to raise plasma concentrations of apoA-I and hence HDL levels are currently intensely studied and include the infusion of lipid-free apoA-I, reconstituted HDL (i.e. CSL-111) or mutant apoA-I [152]. More recently, a compound (RVX-208, Resverlogix) has entered first clinical safety and pharmacokinetic studies, that enhances apoA-I expression in the liver.

Of note, an intravenous infusion of lipid-free apoA-I in men with low HDL cholesterol (<40 mg/dl) has been shown to increase total apoA-I and HDL phospholipid concentration in plasma, without affecting the levels of unesterified or esterified cholesterol in HDL [161]. Moreover, intravenous infusion of apoA-I/phosphatidylcholine disks (reconstituted HDL) has

been demonstrated to increase plasma apoA-I concentration (in particular in small prebeta-migrating particles) and unesterified cholesterol in total HDL [162]. After stopping the infusion, the authors observed an increase in cholesteryl ester content of HDL and large alpha-migrating apoA-I containing HDL, suggesting that the infusion of apoA-I/phosphatidylcholine disks may increase the intravascular production of small pre-beta HDL *in vivo*, and that this may be associated with an increase in the efflux and esterification of unsterified cholesterol [162]. Likewise, in patients with familial hypercholesterolemia the intravenous infusion of human pro-apoA-I (a precursor of apoA-I) liposome complexes led to an increase in plasma levels of apoA-I and HDL, and was associated with an increase in fecal cholesterol excretion (i.e. neutral sterols and bile acids) over a period of 9 days after injection [163].

A small double-blind, randomized, placebo-controlled pilot study by Nissen et al. [164] examined the effects of 5 intravenous injections of recombinant apoA-I $_{\text{Milano}}$ / phospholipid complexes (ETC-216) on coronary atheroma burden in patients with an acute coronary syndrome. ApoA-I $_{\text{Milano}}$ is a rare point mutation in apoA-I that has been identified in a small number of individuals in northern Italy and subjects carrying the apoA-I $_{\text{Milano}}$ mutation display low HDL cholesterol and apoA-I levels, but no clear increase in the risk of cardiovascular disease, that has lead to the suggestion that this may be a particularly vasoprotective variant of apoA-I [165]. Intravenous infusions of recombinant apoA-I$_{\text{Milano}}$/ phospholipid complexes (five doses at weekly intervals) resulted in a small, but significant regression of coronary atheroma volume, as detected by IVUS, compared with no significant change from baseline in the placebo group [164].

Moreover, recently a randomized placebo-controlled trial examined the effects of intravenous infusion of reconstituted HDL on coronary plaque burden as assessed by intravascular ultrasound (IVUS), and included 145 patients that had evaluable serial IVUS examinations

[166]. Patients were randomly assigned to receive 4 weekly infusions of reconstituted HDL (CSL-111, consisting of apoA-I isolated from human plasma and phosphatidylcholine derived from soybean in a molar ratio of 1:150) or placebo [166]. After six weeks of follow-up, the short-term infusion of reconstituted HDL did not result in a significant reduction in percentage change in atheroma volume or nominal change in plaque volume compared with placebo. However, the authors observed a statistically significant improvement in the plaque characterization index [166].

Interestingly, a study from our department was able to demonstrate that intravenous infusion of reconstituted HDL rapidly restored the impaired endothelium-dependent vasodilation in hypercholesterolemic patients, as detected by forearm venous occlusion plethysmography, suggesting a potential therapeutic benefit of this approach in patients at risk for atherosclerosis [53]. Moreover, in 9 subjects with low HDL that were ABCA1 heterozygotes, impaired endothelium-dependent vasodilation was restored by apoA-I/phosphatidylcholine (apoA-I/PC) disks administration [52].

Another approach for raising apoA-I plasma level is that of using apoA-I mimetics, i.e. peptides structurally and functionally related to apoA-I. Numerous studies have suggested that apoA-I mimetics possess atheroprotective effects in animal models, and these compounds are now entering first clinical trials [167, 168].

5. CONCLUSION

Low HDL plasma concentrations are associated with a substantially increased coronary heart disease risk. Over the past years numerous studies have shed light on the mechanisms whereby HDL may exert anti-atherosclerotic effects. In particular, promotion of cholesterol-efflux from lipid loaded macrophages via ABCA1 and ABCG1, anti-inflammatory, anti-thrombotic and endothelial-protective effects of HDL have been identified. At the same time

it has become clear, that the vascular-protective properties of HDL are heterogeneous and may be impaired in patients at high cardiovascular risk. Therefore, HDL-targeted strategies should likely not only increase HDL plasma levels, but also lead to an on-treatment HDL with potent anti-atherosclerotic properties. Finally, successful HDL-targeted therapies may promote regression of atherosclerosis.

Figure Legends

Fig. (1). Potential athero-protective effects of HDL. Besides promoting cholesterol efflux and reverse cholesterol transport from macrophages in the atherosclerotic lesion back to the liver for excretion in the bile, HDL has been suggested to exert important direct endothelial-protective effects. In particular, HDL has been shown to stimulate endothelial nitric oxide (NO) production and endothelium-dependent, NO-mediated vasodilation, to exert anti-oxidant, anti-inflammatory and anti-thrombotic effects in endothelial cells, and to promote endothelial repair processes.

Fig. (2). Endothelial NO synthase (eNOS) activating signaling pathways in endothelial cells stimulated by HDL. HDL has been shown to stimulate eNOS phosphorylation at serine residue 1177 via binding of the major apolipoprotein in HDL, apoA-I, to SR-BI and via binding of HDL-associated lysosphingolipids (SPC, S1P, LSF) to the $S1P_3$ receptor. In addition it has been suggested that HDL increases eNOS protein abundance in endothelial cells by increasing the half-life of eNOS, likely at least in part via Akt/MAP kinase signaling. Moreover, a recent study has suggested that HDL increases eNOS activity in mice on a high-cholesterol diet by promoting endothelial efflux of 7-ketosterol (7-KC) via ABCG-1 and by preserving active eNOS dimer levels.

Fig. (3). Effect of HDL on endothelial cell apoptosis and endothelial repair. Recent studies have suggested that apoA-I may protect endothelial cells against apoptosis induced by TNFα, oxLDL and VLDL. In contrast, HDL-associated lysosphingolipids may confer protection against endothelial cell apoptosis induced by growth factor deprivation. Furthermore, HDL has been demonstrated to stimulate endothelial cell migration either by binding of apoA-I to SR-BI or by binding of sphingosine-1-phosphate to its receptors, S1P1

and S1P3. In addition, recent studies have suggested that HDL improves endothelial repair process, in part by stimulating EPC-mediated endothelial repair capacity.

References

[1] Gordon T, Castelli WP, Hjortland MC, Kannel WB, Dawber TR. High density lipoprotein as a protective factor against coronary heart disease. The Framingham Study. Am J Med 1977; 62: 707-14.

[2] Castelli WP, Garrison RJ, Wilson PW, Abbott RD, Kalousdian S, Kannel WB. Incidence of coronary heart disease and lipoprotein cholesterol levels. The Framingham Study. Jama 1986; 256: 2835-8.

[3] Cullen P, Schulte H, Assmann G. The Munster Heart Study (PROCAM): total mortality in middle-aged men is increased at low total and LDL cholesterol concentrations in smokers but not in nonsmokers. Circulation 1997; 96: 2128-36.

[4] Sharrett AR, Ballantyne CM, Coady SA, Heiss G, Sorlie PD, Catellier D*, et al.* Coronary heart disease prediction from lipoprotein cholesterol levels, triglycerides, lipoprotein(a), apolipoproteins A-I and B, and HDL density subfractions: The Atherosclerosis Risk in Communities (ARIC) Study. Circulation 2001; 104: 1108-13.

[5] Barter P, Gotto AM, LaRosa JC, Maroni J, Szarek M, Grundy SM*, et al.* HDL cholesterol, very low levels of LDL cholesterol, and cardiovascular events. N Engl J Med 2007; 357: 1301-10.

[6] Di Angelantonio E, Sarwar N, Perry P, Kaptoge S, Ray KK, Thompson A*, et al.* Major lipids, apolipoproteins, and risk of vascular disease. Jama 2009; 302: 1993-2000.

[7] Frikke-Schmidt R, Nordestgaard BG, Stene MC, Sethi AA, Remaley AT, Schnohr P*, et al.* Association of loss-of-function mutations in the ABCA1 gene with high-density lipoprotein cholesterol levels and risk of ischemic heart disease. Jama 2008; 299: 2524-32.

[8] Thompson A, Di Angelantonio E, Sarwar N, Erqou S, Saleheen D, Dullaart RP*, et al.* Association of cholesteryl ester transfer protein genotypes with CETP mass and activity, lipid levels, and coronary risk. Jama 2008; 299: 2777-88.

[9] Brunham LR, Kastelein JJ, Hayden MR. ABCA1 gene mutations, HDL cholesterol levels, and risk of ischemic heart disease. Jama 2008; 300: 1997-8; author reply 1998.

[10] Rader DJ. Molecular regulation of HDL metabolism and function: implications for novel therapies. J Clin Invest 2006; 116: 3090-100.

[11] Mineo C, Deguchi H, Griffin JH, Shaul PW. Endothelial and antithrombotic actions of HDL. Circ Res 2006; 98: 1352-64.

[12] Barter PJ, Nicholls S, Rye KA, Anantharamaiah GM, Navab M, Fogelman AM. Antiinflammatory properties of HDL. Circ Res 2004; 95: 764-72.

[13] Charakida M, Besler C, Batuca JR, Sangle S, Marques S, Sousa M*, et al.* Vascular abnormalities, paraoxonase activity, and dysfunctional HDL in primary antiphospholipid syndrome. Jama 2009; 302: 1210-7.

[14] Sorrentino SA, Besler C, Rohrer L, Meyer M, Heinrich K, Bahlmann F*, et al.* Endothelial-vasoprotective effects of HDL are impaired in patients with type-2 diabetes, but are improved after extended-release niacin therapy. Circulation 2009: in press.

[15] Cuchel M, Rader DJ. Macrophage reverse cholesterol transport: key to the regression of atherosclerosis? Circulation 2006; 113: 2548-55.

[16] Glomset JA. The plasma lecithins:cholesterol acyltransferase reaction. J Lipid Res 1968; 9: 155-67.

[17] Tall AR, Costet P, Wang N. Regulation and mechanisms of macrophage cholesterol efflux. J Clin Invest 2002; 110: 899-904.

[18] Tall AR, Yvan-Charvet L, Terasaka N, Pagler T, Wang N. HDL, ABC transporters, and cholesterol efflux: implications for the treatment of atherosclerosis. Cell Metab 2008; 7: 365-75.

[19] Bortnick AE, Rothblat GH, Stoudt G, Hoppe KL, Royer LJ, McNeish J*, et al.* The correlation of ATP-binding cassette 1 mRNA levels with cholesterol efflux from various cell lines. J Biol Chem 2000; 275: 28634-40.

[20] Haghpassand M, Bourassa PA, Francone OL, Aiello RJ. Monocyte/macrophage expression of ABCA1 has minimal contribution to plasma HDL levels. J Clin Invest 2001; 108: 1315-20.

[21] Van Eck M, Singaraja RR, Ye D, Hildebrand RB, James ER, Hayden MR*, et al.* Macrophage ATP-binding cassette transporter A1 overexpression inhibits atherosclerotic lesion progression in low-density lipoprotein receptor knockout mice. Arterioscler Thromb Vasc Biol 2006; 26: 929-34.

[22] Aiello RJ, Brees D, Bourassa PA, Royer L, Lindsey S, Coskran T*, et al.* Increased atherosclerosis in hyperlipidemic mice with inactivation of ABCA1 in macrophages. Arterioscler Thromb Vasc Biol 2002; 22: 630-7.

[23] Groen AK, Bloks VW, Bandsma RH, Ottenhoff R, Chimini G, Kuipers F. Hepatobiliary cholesterol transport is not impaired in Abca1-null mice lacking HDL. J Clin Invest 2001; 108: 843-50.

[24] Wang MD, Kiss RS, Franklin V, McBride HM, Whitman SC, Marcel YL. Different cellular traffic of LDL-cholesterol and acetylated LDL-cholesterol leads to distinct reverse cholesterol transport pathways. J Lipid Res 2007; 48: 633-45.

[25] Kennedy MA, Barrera GC, Nakamura K, Baldan A, Tarr P, Fishbein MC*, et al.* ABCG1 has a critical role in mediating cholesterol efflux to HDL and preventing cellular lipid accumulation. Cell Metab 2005; 1: 121-31.

[26] Out R, Hoekstra M, Meurs I, de Vos P, Kuiper J, Van Eck M*, et al.* Total body ABCG1 expression protects against early atherosclerotic lesion development in mice. Arterioscler Thromb Vasc Biol 2007; 27: 594-9.

[27] Baldan A, Pei L, Lee R, Tarr P, Tangirala RK, Weinstein MM*, et al.* Impaired development of atherosclerosis in hyperlipidemic Ldlr-/- and ApoE-/- mice transplanted with Abcg1-/- bone marrow. Arterioscler Thromb Vasc Biol 2006; 26: 2301-7.

[28] Ranalletta M, Wang N, Han S, Yvan-Charvet L, Welch C, Tall AR. Decreased atherosclerosis in low-density lipoprotein receptor knockout mice transplanted with Abcg1-/- bone marrow. Arterioscler Thromb Vasc Biol 2006; 26: 2308-15.

[29] Wang X, Rader DJ. Molecular regulation of macrophage reverse cholesterol transport. Curr Opin Cardiol 2007; 22: 368-72.

[30] Wang X, Collins HL, Ranalletta M, Fuki IV, Billheimer JT, Rothblat GH*, et al.* Macrophage ABCA1 and ABCG1, but not SR-BI, promote macrophage reverse cholesterol transport in vivo. J Clin Invest 2007; 117: 2216-24.

[31] Yvan-Charvet L, Ranalletta M, Wang N, Han S, Terasaka N, Li R*, et al.* Combined deficiency of ABCA1 and ABCG1 promotes foam cell accumulation and accelerates atherosclerosis in mice. J Clin Invest 2007; 117: 3900-8.

[32] Yvan-Charvet L, Pagler TA, Wang N, Senokuchi T, Brundert M, Li H*, et al.* SR-BI inhibits ABCG1-stimulated net cholesterol efflux from cells to plasma HDL. J Lipid Res 2008; 49: 107-14.

[33] Covey SD, Krieger M, Wang W, Penman M, Trigatti BL. Scavenger receptor class B type I-mediated protection against atherosclerosis in LDL receptor-negative mice involves its expression in bone marrow-derived cells. Arterioscler Thromb Vasc Biol 2003; 23: 1589-94.

[34] Zhang W, Yancey PG, Su YR, Babaev VR, Zhang Y, Fazio S*, et al.* Inactivation of macrophage scavenger receptor class B type I promotes atherosclerotic lesion development in apolipoprotein E-deficient mice. Circulation 2003; 108: 2258-63.

[35] Rader DJ. Lecithin: cholesterol acyltransferase and atherosclerosis: another high-density lipoprotein story that doesn't quite follow the script. Circulation 2009; 120: 549-52.

[36] Calabresi L, Favari E, Moleri E, Adorni MP, Pedrelli M, Costa S*, et al.* Functional LCAT is not required for macrophage cholesterol efflux to human serum. Atherosclerosis 2009; 204: 141-6.

[37] Tanigawa H, Billheimer JT, Tohyama J, Fuki IV, Ng DS, Rothblat GH*, et al.* Lecithin: cholesterol acyltransferase expression has minimal effects on macrophage reverse cholesterol transport in vivo. Circulation 2009; 120: 160-9.

[38] Schwartz CC, Halloran LG, Vlahcevic ZR, Gregory DH, Swell L. Preferential utilization of free cholesterol from high-density lipoproteins for biliary cholesterol secretion in man. Science 1978; 200: 62-4.

[39] Schwartz CC, Berman M, Vlahcevic ZR, Swell L. Multicompartmental analysis of cholesterol metabolism in man. Quantitative kinetic evaluation of precursor sources and turnover of high density lipoprotein cholesterol esters. J Clin Invest 1982; 70: 863-76.

[40] Calabresi L, Baldassarre D, Castelnuovo S, Conca P, Bocchi L, Candini C*, et al.* Functional lecithin: cholesterol acyltransferase is not required for efficient atheroprotection in humans. Circulation 2009; 120: 628-35.

[41] Ji Y, Wang N, Ramakrishnan R, Sehayek E, Huszar D, Breslow JL*, et al.* Hepatic scavenger receptor BI promotes rapid clearance of high density lipoprotein free cholesterol and its transport into bile. J Biol Chem 1999; 274: 33398-402.

[42] Zhang Y, Da Silva JR, Reilly M, Billheimer JT, Rothblat GH, Rader DJ. Hepatic expression of scavenger receptor class B type I (SR-BI) is a positive regulator of macrophage reverse cholesterol transport in vivo. J Clin Invest 2005; 115: 2870-4.

[43] Tanigawa H, Billheimer JT, Tohyama J, Zhang Y, Rothblat G, Rader DJ. Expression of cholesteryl ester transfer protein in mice promotes macrophage reverse cholesterol transport. Circulation 2007; 116: 1267-73.

[44] Matsuura F, Wang N, Chen W, Jiang XC, Tall AR. HDL from CETP-deficient subjects shows enhanced ability to promote cholesterol efflux from macrophages in an apoE- and ABCG1-dependent pathway. J Clin Invest 2006; 116: 1435-42.

[45] Landmesser U, Hornig B, Drexler H. Endothelial function: a critical determinant in atherosclerosis? Circulation 2004; 109: II27-33.

[46] Moncada S, Higgs A. The L-arginine-nitric oxide pathway. N Engl J Med 1993; 329: 2002-12.

[47] Yuhanna IS, Zhu Y, Cox BE, Hahner LD, Osborne-Lawrence S, Lu P*, et al.* High-density lipoprotein binding to scavenger receptor-BI activates endothelial nitric oxide synthase. Nat Med 2001; 7: 853-7.

[48] Kuvin JT, Ramet ME, Patel AR, Pandian NG, Mendelsohn ME, Karas RH. A novel mechanism for the beneficial vascular effects of high-density lipoprotein cholesterol: enhanced vasorelaxation and increased endothelial nitric oxide synthase expression. Am Heart J 2002; 144: 165-72.

[49] Mineo C, Yuhanna IS, Quon MJ, Shaul PW. High density lipoprotein-induced endothelial nitric-oxide synthase activation is mediated by Akt and MAP kinases. J Biol Chem 2003; 278: 9142-9.

[50] Ramet ME, Ramet M, Lu Q, Nickerson M, Savolainen MJ, Malzone A*, et al.* High-density lipoprotein increases the abundance of eNOS protein in human vascular endothelial cells by increasing its half-life. J Am Coll Cardiol 2003; 41: 2288-97.

[51] Nofer JR, van der Giet M, Tolle M, Wolinska I, von Wnuck Lipinski K, Baba HA*, et al.* HDL induces NO-dependent vasorelaxation via the lysophospholipid receptor S1P3. J Clin Invest 2004; 113: 569-81.

[52] Bisoendial RJ, Hovingh GK, Levels JH, Lerch PG, Andresen I, Hayden MR*, et al.* Restoration of endothelial function by increasing high-density lipoprotein in subjects with isolated low high-density lipoprotein. Circulation 2003; 107: 2944-8.

[53] Spieker LE, Sudano I, Hurlimann D, Lerch PG, Lang MG, Binggeli C*, et al.* High-density lipoprotein restores endothelial function in hypercholesterolemic men. Circulation 2002; 105: 1399-402.

[54] Uittenbogaard A, Shaul PW, Yuhanna IS, Blair A, Smart EJ. High density lipoprotein prevents oxidized low density lipoprotein-induced inhibition of endothelial nitric-oxide synthase localization and activation in caveolae. J Biol Chem 2000; 275: 11278-83.

[55] Dimmeler S, Fleming I, Fisslthaler B, Hermann C, Busse R, Zeiher AM. Activation of nitric oxide synthase in endothelial cells by Akt-dependent phosphorylation. Nature 1999; 399: 601-5.

[56] Shaul PW, Mineo C. HDL action on the vascular wall: is the answer NO? J Clin Invest 2004; 113: 509-13.

[57] Terasaka N, Yu S, Yvan-Charvet L, Wang N, Mzhavia N, Langlois R*, et al.* ABCG1 and HDL protect against endothelial dysfunction in mice fed a high-cholesterol diet. J Clin Invest 2008; 118: 3701-13.

[58] Hansson GK. Inflammation, atherosclerosis, and coronary artery disease. N Engl J Med 2005; 352: 1685-95.

[59] Navab M, Ananthramaiah GM, Reddy ST, Van Lenten BJ, Ansell BJ, Fonarow GC, *et al.* The oxidation hypothesis of atherogenesis: the role of oxidized phospholipids and HDL. J Lipid Res 2004; 45: 993-1007.

[60] Shao B, Heinecke JW. HDL, lipid peroxidation, and atherosclerosis. J Lipid Res 2009; 50: 599-601.

[61] Navab M, Hama SY, Cooke CJ, Anantharamaiah GM, Chaddha M, Jin L, *et al.* Normal high density lipoprotein inhibits three steps in the formation of mildly oxidized low density lipoprotein: step 1. J Lipid Res 2000; 41: 1481-94.

[62] Navab M, Hama SY, Anantharamaiah GM, Hassan K, Hough GP, Watson AD, *et al.* Normal high density lipoprotein inhibits three steps in the formation of mildly oxidized low density lipoprotein: steps 2 and 3. J Lipid Res 2000; 41: 1495-508.

[63] Bowry VW, Stanley KK, Stocker R. High density lipoprotein is the major carrier of lipid hydroperoxides in human blood plasma from fasting donors. Proc Natl Acad Sci U S A 1992; 89: 10316-20.

[64] Proudfoot JM, Barden AE, Loke WM, Croft KD, Puddey IB, Mori TA. HDL is the major lipoprotein carrier of plasma F2-isoprostanes. J Lipid Res 2009; 50: 716-22.

[65] Navab M, Berliner JA, Subbanagounder G, Hama S, Lusis AJ, Castellani LW, *et al.* HDL and the inflammatory response induced by LDL-derived oxidized phospholipids. Arterioscler Thromb Vasc Biol 2001; 21: 481-8.

[66] Shih DM, Gu L, Xia YR, Navab M, Li WF, Hama S, *et al.* Mice lacking serum paraoxonase are susceptible to organophosphate toxicity and atherosclerosis. Nature 1998; 394: 284-7.

[67] Tward A, Xia YR, Wang XP, Shi YS, Park C, Castellani LW*, et al.* Decreased atherosclerotic lesion formation in human serum paraoxonase transgenic mice. Circulation 2002; 106: 484-90.

[68] Terasaka N, Wang N, Yvan-Charvet L, Tall AR. High-density lipoprotein protects macrophages from oxidized low-density lipoprotein-induced apoptosis by promoting efflux of 7-ketocholesterol via ABCG1. Proc Natl Acad Sci U S A 2007; 104: 15093-8.

[69] Nicholls SJ, Dusting GJ, Cutri B, Bao S, Drummond GR, Rye KA*, et al.* Reconstituted high-density lipoproteins inhibit the acute pro-oxidant and proinflammatory vascular changes induced by a periarterial collar in normocholesterolemic rabbits. Circulation 2005; 111: 1543-50.

[70] Van Linthout S, Spillmann F, Lorenz M, Meloni M, Jacobs F, Egorova M*, et al.* Vascular-protective effects of high-density lipoprotein include the downregulation of the angiotensin II type 1 receptor. Hypertension 2009; 53: 682-7.

[71] Navab M, Imes SS, Hama SY, Hough GP, Ross LA, Bork RW*, et al.* Monocyte transmigration induced by modification of low density lipoprotein in cocultures of human aortic wall cells is due to induction of monocyte chemotactic protein 1 synthesis and is abolished by high density lipoprotein. J Clin Invest 1991; 88: 2039-46.

[72] Mackness B, Hine D, Liu Y, Mastorikou M, Mackness M. Paraoxonase-1 inhibits oxidised LDL-induced MCP-1 production by endothelial cells. Biochem Biophys Res Commun 2004; 318: 680-3.

[73] Cockerill GW, Rye KA, Gamble JR, Vadas MA, Barter PJ. High-density lipoproteins inhibit cytokine-induced expression of endothelial cell adhesion molecules. Arterioscler Thromb Vasc Biol 1995; 15: 1987-94.

[74] Calabresi L, Franceschini G, Sirtori CR, De Palma A, Saresella M, Ferrante P*, et al.* Inhibition of VCAM-1 expression in endothelial cells by reconstituted high density lipoproteins. Biochem Biophys Res Commun 1997; 238: 61-5.

[75] Xia P, Vadas MA, Rye KA, Barter PJ, Gamble JR. High density lipoproteins (HDL) interrupt the sphingosine kinase signaling pathway. A possible mechanism for protection against atherosclerosis by HDL. J Biol Chem 1999; 274: 33143-7.

[76] Park SH, Park JH, Kang JS, Kang YH. Involvement of transcription factors in plasma HDL protection against TNF-alpha-induced vascular cell adhesion molecule-1 expression. Int J Biochem Cell Biol 2003; 35: 168-82.

[77] Maier JA, Barenghi L, Pagani F, Bradamante S, Comi P, Ragnotti G. The protective role of high-density lipoprotein on oxidized-low-density-lipoprotein-induced U937/endothelial cell interactions. Eur J Biochem 1994; 221: 35-41.

[78] Dimayuga P, Zhu J, Oguchi S, Chyu KY, Xu XO, Yano J*, et al.* Reconstituted HDL containing human apolipoprotein A-1 reduces VCAM-1 expression and neointima formation following periadventitial cuff-induced carotid injury in apoE null mice. Biochem Biophys Res Commun 1999; 264: 465-8.

[79] Van Linthout S, Spillmann F, Riad A, Trimpert C, Lievens J, Meloni M*, et al.* Human apolipoprotein A-I gene transfer reduces the development of experimental diabetic cardiomyopathy. Circulation 2008; 117: 1563-73.

[80] Dansky HM, Charlton SA, Barlow CB, Tamminen M, Smith JD, Frank JS*, et al.* Apo A-I inhibits foam cell formation in Apo E-deficient mice after monocyte adherence to endothelium. J Clin Invest 1999; 104: 31-9.

[81] Ashby DT, Rye KA, Clay MA, Vadas MA, Gamble JR, Barter PJ. Factors influencing the ability of HDL to inhibit expression of vascular cell adhesion molecule-1 in endothelial cells. Arterioscler Thromb Vasc Biol 1998; 18: 1450-5.

[82] Van Lenten BJ, Hama SY, de Beer FC, Stafforini DM, McIntyre TM, Prescott SM*, et al.* Anti-inflammatory HDL becomes pro-inflammatory during the acute phase response. Loss of protective effect of HDL against LDL oxidation in aortic wall cell cocultures. J Clin Invest 1995; 96: 2758-67.

[83] Patel S, Drew BG, Nakhla S, Duffy SJ, Murphy AJ, Barter PJ*, et al.* Reconstituted high-density lipoprotein increases plasma high-density lipoprotein anti-inflammatory properties and cholesterol efflux capacity in patients with type 2 diabetes. J Am Coll Cardiol 2009; 53: 962-71.

[84] Baker PW, Rye KA, Gamble JR, Vadas MA, Barter PJ. Phospholipid composition of reconstituted high density lipoproteins influences their ability to inhibit endothelial cell adhesion molecule expression. J Lipid Res 2000; 41: 1261-7.

[85] Collins T, Cybulsky MI. NF-kappaB: pivotal mediator or innocent bystander in atherogenesis? J Clin Invest 2001; 107: 255-64.

[86] Besler C, Doerries C, Giannotti G, Luscher TF, Landmesser U. Pharmacological approaches to improve endothelial repair mechanisms. Expert Rev Cardiovasc Ther 2008; 6: 1071-82.

[87] Ross R. The pathogenesis of atherosclerosis: a perspective for the 1990s. Nature 1993; 362: 801-9.

[88] Sugano M, Tsuchida K, Makino N. High-density lipoproteins protect endothelial cells from tumor necrosis factor-alpha-induced apoptosis. Biochem Biophys Res Commun 2000; 272: 872-6.

[89] Suc I, Escargueil-Blanc I, Troly M, Salvayre R, Negre-Salvayre A. HDL and ApoA prevent cell death of endothelial cells induced by oxidized LDL. Arterioscler Thromb Vasc Biol 1997; 17: 2158-66.

[90] Nofer JR, Levkau B, Wolinska I, Junker R, Fobker M, von Eckardstein A*, et al.* Suppression of endothelial cell apoptosis by high density lipoproteins (HDL) and HDL-associated lysosphingolipids. J Biol Chem 2001; 276: 34480-5.

[91] Speidel MT, Booyse FM, Abrams A, Moore MA, Chung BH. Lipolyzed hypertriglyceridemic serum and triglyceride-rich lipoprotein cause lipid accumulation in and are cytotoxic to cultured human endothelial cells. High density lipoproteins inhibit this cytotoxicity. Thromb Res 1990; 58: 251-64.

[92] de Souza JA, Vindis C, Negre-Salvayre A, Rye KA, Couturier M, Therond P*, et al.* Small, dense HDL3 particles attenuates apoptosis in endothelial cells: Pivotal role of apolipoprotein A-I. J Cell Mol Med 2009.

[93] Kimura T, Sato K, Kuwabara A, Tomura H, Ishiwara M, Kobayashi I*, et al.* Sphingosine 1-phosphate may be a major component of plasma lipoproteins responsible for the cytoprotective actions in human umbilical vein endothelial cells. J Biol Chem 2001; 276: 31780-5.

[94] Kimura T, Sato K, Malchinkhuu E, Tomura H, Tamama K, Kuwabara A*, et al.* High-density lipoprotein stimulates endothelial cell migration and survival through sphingosine 1-phosphate and its receptors. Arterioscler Thromb Vasc Biol 2003; 23: 1283-8.

[95] Kontush A, Therond P, Zerrad A, Couturier M, Negre-Salvayre A, de Souza JA*, et al.* Preferential sphingosine-1-phosphate enrichment and sphingomyelin depletion are key features of small dense HDL3 particles: relevance to antiapoptotic and antioxidative activities. Arterioscler Thromb Vasc Biol 2007; 27: 1843-9.

[96] Caplan BA, Schwartz CJ. Increased endothelial cell turnover in areas of in vivo Evans Blue uptake in the pig aorta. Atherosclerosis 1973; 17: 401-17.

[97] Sorrentino SA, Bahlmann FH, Besler C, Muller M, Schulz S, Kirchhoff N*, et al.* Oxidant stress impairs in vivo reendothelialization capacity of endothelial progenitor cells from patients with type 2 diabetes mellitus: restoration by the peroxisome proliferator-activated receptor-gamma agonist rosiglitazone. Circulation 2007; 116: 163-73.

[98] Walter DH, Rittig K, Bahlmann FH, Kirchmair R, Silver M, Murayama T*, et al.* Statin therapy accelerates reendothelialization: a novel effect involving mobilization and incorporation of bone marrow-derived endothelial progenitor cells. Circulation 2002; 105: 3017-24.

[99] Werner N, Priller J, Laufs U, Endres M, Bohm M, Dirnagl U*, et al.* Bone marrow-derived progenitor cells modulate vascular reendothelialization and neointimal formation: effect of 3-hydroxy-3-methylglutaryl coenzyme a reductase inhibition. Arterioscler Thromb Vasc Biol 2002; 22: 1567-72.

[100] Foteinos G, Hu Y, Xiao Q, Metzler B, Xu Q. Rapid endothelial turnover in atherosclerosis-prone areas coincides with stem cell repair in apolipoprotein E-deficient mice. Circulation 2008; 117: 1856-63.

[101] Wassmann S, Werner N, Czech T, Nickenig G. Improvement of endothelial function by systemic transfusion of vascular progenitor cells. Circ Res 2006; 99: e74-83.

[102] Seetharam D, Mineo C, Gormley AK, Gibson LL, Vongpatanasin W, Chambliss KL*, et al.* High-density lipoprotein promotes endothelial cell migration and reendothelialization via scavenger receptor-B type I. Circ Res 2006; 98: 63-72.

[103] Zhu W, Saddar S, Seetharam D, Chambliss KL, Longoria C, Silver DL*, et al.* The scavenger receptor class B type I adaptor protein PDZK1 maintains endothelial monolayer integrity. Circ Res 2008; 102: 480-7.

[104] Murugesan G, Sa G, Fox PL. High-density lipoprotein stimulates endothelial cell movement by a mechanism distinct from basic fibroblast growth factor. Circ Res 1994; 74: 1149-56.

[105] Miura S, Tanigawa H, Matsuo Y, Fujino M, Kawamura A, Saku K. Ras/Raf1-dependent signal in sphingosine-1-phosphate-induced tube formation in human coronary artery endothelial cells. Biochem Biophys Res Commun 2003; 306: 924-9.

[106] Feng Y, Jacobs F, Van Craeyveld E, Brunaud C, Snoeys J, Tjwa M*, et al.* Human ApoA-I transfer attenuates transplant arteriosclerosis via enhanced incorporation of bone marrow-derived endothelial progenitor cells. Arterioscler Thromb Vasc Biol 2008; 28: 278-83.

[107] Feng Y, van Eck M, Van Craeyveld E, Jacobs F, Carlier V, Van Linthout S*, et al.* Critical role of scavenger receptor-BI-expressing bone marrow-derived endothelial progenitor cells in the attenuation of allograft vasculopathy after human apo A-I transfer. Blood 2009; 113: 755-64.

[108] Tso C, Martinic G, Fan WH, Rogers C, Rye KA, Barter PJ. High-density lipoproteins enhance progenitor-mediated endothelium repair in mice. Arterioscler Thromb Vasc Biol 2006; 26: 1144-9.

[109] Sumi M, Sata M, Miura S, Rye KA, Toya N, Kanaoka Y*, et al.* Reconstituted high-density lipoprotein stimulates differentiation of endothelial progenitor cells and enhances ischemia-induced angiogenesis. Arterioscler Thromb Vasc Biol 2007; 27: 813-8.

[110] Navab M, Anantharamaiah GM, Reddy ST, Van Lenten BJ, Ansell BJ, Fogelman AM. Mechanisms of disease: proatherogenic HDL--an evolving field. Nat Clin Pract Endocrinol Metab 2006; 2: 504-11.

[111] Zheng L, Nukuna B, Brennan ML, Sun M, Goormastic M, Settle M*, et al.* Apolipoprotein A-I is a selective target for myeloperoxidase-catalyzed oxidation and functional impairment in subjects with cardiovascular disease. J Clin Invest 2004; 114: 529-41.

[112] Shao B, Cavigiolio G, Brot N, Oda MN, Heinecke JW. Methionine oxidation impairs reverse cholesterol transport by apolipoprotein A-I. Proc Natl Acad Sci U S A 2008; 105: 12224-9.

[113] Wu Z, Wagner MA, Zheng L, Parks JS, Shy JM, 3rd, Smith JD*, et al.* The refined structure of nascent HDL reveals a key functional domain for particle maturation and dysfunction. Nat Struct Mol Biol 2007; 14: 861-8.

[114] Undurti A, Huang Y, Lupica JA, Smith JD, DiDonato JA, Hazen SL. Modification of high density lipoprotein by myeloperoxidase generates a pro-inflammatory particle. J Biol Chem 2009; 284: 30825-35.

[115] Kontush A, Chapman MJ. Functionally defective high-density lipoprotein: a new therapeutic target at the crossroads of dyslipidemia, inflammation, and atherosclerosis. Pharmacol Rev 2006; 58: 342-74.

[116] Baker PW, Rye KA, Gamble JR, Vadas MA, Barter PJ. Ability of reconstituted high density lipoproteins to inhibit cytokine-induced expression of vascular cell adhesion molecule-1 in human umbilical vein endothelial cells. J Lipid Res 1999; 40: 345-53.

[117] McMahon M, Grossman J, FitzGerald J, Dahlin-Lee E, Wallace DJ, Thong BY*, et al.* Proinflammatory high-density lipoprotein as a biomarker for atherosclerosis in patients with systemic lupus erythematosus and rheumatoid arthritis. Arthritis Rheum 2006; 54: 2541-9.

[118] Weihrauch D, Xu H, Shi Y, Wang J, Brien J, Jones DW*, et al.* Effects of D-4F on vasodilation, oxidative stress, angiostatin, myocardial inflammation, and angiogenic potential in tight-skin mice. Am J Physiol Heart Circ Physiol 2007; 293: H1432-41.

[119] de Souza JA, Vindis C, Hansel B, Negre-Salvayre A, Therond P, Serrano CV, Jr.*, et al.* Metabolic syndrome features small, apolipoprotein A-I-poor, triglyceride-rich HDL3 particles with defective anti-apoptotic activity. Atherosclerosis 2008; 197: 84-94.

[120] Persegol L, Verges B, Foissac M, Gambert P, Duvillard L. Inability of HDL from type 2 diabetic patients to counteract the inhibitory effect of oxidised LDL on endothelium-dependent vasorelaxation. Diabetologia 2006; 49: 1380-6.

[121] Ansell BJ, Navab M, Hama S, Kamranpour N, Fonarow G, Hough G*, et al.* Inflammatory/antiinflammatory properties of high-density lipoprotein distinguish patients from control subjects better than high-density lipoprotein cholesterol levels and are favorably affected by simvastatin treatment. Circulation 2003; 108: 2751-6.

[122] Kalantar-Zadeh K, Kopple JD, Kamranpour N, Fogelman AM, Navab M. HDL-inflammatory index correlates with poor outcome in hemodialysis patients. Kidney Int 2007; 72: 1149-56.

[123] Altschul R, Hoffer A, Stephen JD. Influence of nicotinic acid on serum cholesterol in man. Arch Biochem 1955; 54: 558-9.

[124] Gille A, Bodor ET, Ahmed K, Offermanns S. Nicotinic acid: pharmacological effects and mechanisms of action. Annu Rev Pharmacol Toxicol 2008; 48: 79-106.

[125]. Clofibrate and niacin in coronary heart disease. Jama 1975; 231: 360-81.

[126] Canner PL, Berge KG, Wenger NK, Stamler J, Friedman L, Prineas RJ*, et al.* Fifteen year mortality in Coronary Drug Project patients: long-term benefit with niacin. J Am Coll Cardiol 1986; 8: 1245-55.

[127] Capuzzi DM, Guyton JR, Morgan JM, Goldberg AC, Kreisberg RA, Brusco OA*, et al.* Efficacy and safety of an extended-release niacin (Niaspan): a long-term study. Am J Cardiol 1998; 82: 74U-81U; discussion 85U-86U.

[128] Guyton JR, Blazing MA, Hagar J, Kashyap ML, Knopp RH, McKenney JM*, et al.* Extended-release niacin vs gemfibrozil for the treatment of low levels of high-density lipoprotein cholesterol. Niaspan-Gemfibrozil Study Group. Arch Intern Med 2000; 160: 1177-84.

[129] Grundy SM, Vega GL, McGovern ME, Tulloch BR, Kendall DM, Fitz-Patrick D*, et al.* Efficacy, safety, and tolerability of once-daily niacin for the treatment of dyslipidemia associated with type 2 diabetes: results of the assessment of diabetes control and evaluation of the efficacy of niaspan trial. Arch Intern Med 2002; 162: 1568-76.

[130] Warnholtz A, Wild P, Ostad MA, Elsner V, Stieber F, Schinzel R*, et al.* Effects of oral niacin on endothelial dysfunction in patients with coronary artery disease: results of the randomized, double-blind, placebo-controlled INEF study. Atherosclerosis 2009; 204: 216-21.

[131] Benjo AM, Maranhao RC, Coimbra SR, Andrade AC, Favarato D, Molina MS*, et al.* Accumulation of chylomicron remnants and impaired vascular reactivity occur in subjects with isolated low HDL cholesterol: effects of niacin treatment. Atherosclerosis 2006; 187: 116-22.

[132] Shao B, Oda MN, Oram JF, Heinecke JW. Myeloperoxidase: an inflammatory enzyme for generating dysfunctional high density lipoprotein. Curr Opin Cardiol 2006; 21: 322-8.

[133] Brown BG, Zhao XQ, Chait A, Fisher LD, Cheung MC, Morse JS*, et al.* Simvastatin and niacin, antioxidant vitamins, or the combination for the prevention of coronary disease. N Engl J Med 2001; 345: 1583-92.

[134] Taylor AJ, Sullenberger LE, Lee HJ, Lee JK, Grace KA. Arterial Biology for the Investigation of the Treatment Effects of Reducing Cholesterol (ARBITER) 2: a double-blind, placebo-controlled study of extended-release niacin on atherosclerosis progression in secondary prevention patients treated with statins. Circulation 2004; 110: 3512-7.

[135] Taylor AJ, Lee HJ, Sullenberger LE. The effect of 24 months of combination statin and extended-release niacin on carotid intima-media thickness: ARBITER 3. Curr Med Res Opin 2006; 22: 2243-50.

[136] Lee JM, Robson MD, Yu LM, Shirodaria CC, Cunnington C, Kylintireas I*, et al.* Effects of high-dose modified-release nicotinic acid on atherosclerosis and vascular function: a randomized, placebo-controlled, magnetic resonance imaging study. J Am Coll Cardiol 2009; 54: 1787-94.

[137] Taylor AJ, Villines TC, Stanek EJ, Devine PJ, Griffen L, Miller M*, et al.* Extended-release niacin or ezetimibe and carotid intima-media thickness. N Engl J Med 2009; 361: 2113-22.

[138] Tall AR. CETP inhibitors to increase HDL cholesterol levels. N Engl J Med 2007; 356: 1364-6.

[139] Masson D, Jiang XC, Lagrost L, Tall AR. The role of plasma lipid transfer proteins in lipoprotein metabolism and atherogenesis. J Lipid Res 2009; 50 Suppl: S201-6.

[140] Brousseau ME, Diffenderfer MR, Millar JS, Nartsupha C, Asztalos BF, Welty FK*, et al.* Effects of cholesteryl ester transfer protein inhibition on high-density lipoprotein

subspecies, apolipoprotein A-I metabolism, and fecal sterol excretion. Arterioscler Thromb Vasc Biol 2005; 25: 1057-64.

[141] Marotti KR, Castle CK, Boyle TP, Lin AH, Murray RW, Melchior GW. Severe atherosclerosis in transgenic mice expressing simian cholesteryl ester transfer protein. Nature 1993; 364: 73-5.

[142] Plump AS, Masucci-Magoulas L, Bruce C, Bisgaier CL, Breslow JL, Tall AR. Increased atherosclerosis in ApoE and LDL receptor gene knock-out mice as a result of human cholesteryl ester transfer protein transgene expression. Arterioscler Thromb Vasc Biol 1999; 19: 1105-10.

[143] Westerterp M, van der Hoogt CC, de Haan W, Offerman EH, Dallinga-Thie GM, Jukema JW*, et al.* Cholesteryl ester transfer protein decreases high-density lipoprotein and severely aggravates atherosclerosis in APOE*3-Leiden mice. Arterioscler Thromb Vasc Biol 2006; 26: 2552-9.

[144] Hayek T, Masucci-Magoulas L, Jiang X, Walsh A, Rubin E, Breslow JL*, et al.* Decreased early atherosclerotic lesions in hypertriglyceridemic mice expressing cholesteryl ester transfer protein transgene. J Clin Invest 1995; 96: 2071-4.

[145] Brown ML, Inazu A, Hesler CB, Agellon LB, Mann C, Whitlock ME*, et al.* Molecular basis of lipid transfer protein deficiency in a family with increased high-density lipoproteins. Nature 1989; 342: 448-51.

[146] Inazu A, Brown ML, Hesler CB, Agellon LB, Koizumi J, Takata K*, et al.* Increased high-density lipoprotein levels caused by a common cholesteryl-ester transfer protein gene mutation. N Engl J Med 1990; 323: 1234-8.

[147] Zhong S, Sharp DS, Grove JS, Bruce C, Yano K, Curb JD*, et al.* Increased coronary heart disease in Japanese-American men with mutation in the cholesteryl ester transfer protein gene despite increased HDL levels. J Clin Invest 1996; 97: 2917-23.

[148] Curb JD, Abbott RD, Rodriguez BL, Masaki K, Chen R, Sharp DS*, et al.* A prospective study of HDL-C and cholesteryl ester transfer protein gene mutations and the risk of coronary heart disease in the elderly. J Lipid Res 2004; 45: 948-53.

[149] Boekholdt SM, Sacks FM, Jukema JW, Shepherd J, Freeman DJ, McMahon AD*, et al.* Cholesteryl ester transfer protein TaqIB variant, high-density lipoprotein cholesterol levels, cardiovascular risk, and efficacy of pravastatin treatment: individual patient meta-analysis of 13,677 subjects. Circulation 2005; 111: 278-87.

[150] Kastelein JJ, van Leuven SI, Burgess L, Evans GW, Kuivenhoven JA, Barter PJ*, et al.* Effect of torcetrapib on carotid atherosclerosis in familial hypercholesterolemia. N Engl J Med 2007; 356: 1620-30.

[151] Barter PJ, Caulfield M, Eriksson M, Grundy SM, Kastelein JJ, Komajda M*, et al.* Effects of torcetrapib in patients at high risk for coronary events. N Engl J Med 2007; 357: 2109-22.

[152] Duffy D, Rader DJ. Update on strategies to increase HDL quantity and function. Nat Rev Cardiol 2009; 6: 455-63.

[153] Krishna R, Anderson MS, Bergman AJ, Jin B, Fallon M, Cote J*, et al.* Effect of the cholesteryl ester transfer protein inhibitor, anacetrapib, on lipoproteins in patients with dyslipidaemia and on 24-h ambulatory blood pressure in healthy individuals: two double-blind, randomised placebo-controlled phase I studies. Lancet 2007; 370: 1907-14.

[154] Bloomfield D, Carlson GL, Sapre A, Tribble D, McKenney JM, Littlejohn TW, 3rd*, et al.* Efficacy and safety of the cholesteryl ester transfer protein inhibitor anacetrapib as monotherapy and coadministered with atorvastatin in dyslipidemic patients. Am Heart J 2009; 157: 352-360 e2.

[155] Yvan-Charvet L, Matsuura F, Wang N, Bamberger MJ, Nguyen T, Rinninger F*, et al.* Inhibition of cholesteryl ester transfer protein by torcetrapib modestly increases macrophage cholesterol efflux to HDL. Arterioscler Thromb Vasc Biol 2007; 27: 1132-8.

[156] Catalano G, Julia Z, Frisdal E, Vedie B, Fournier N, Le Goff W*, et al.* Torcetrapib differentially modulates the biological activities of HDL2 and HDL3 particles in the reverse cholesterol transport pathway. Arterioscler Thromb Vasc Biol 2009; 29: 268-75.

[157] Plump AS, Scott CJ, Breslow JL. Human apolipoprotein A-I gene expression increases high density lipoprotein and suppresses atherosclerosis in the apolipoprotein E-deficient mouse. Proc Natl Acad Sci U S A 1994; 91: 9607-11.

[158] Tangirala RK, Tsukamoto K, Chun SH, Usher D, Pure E, Rader DJ. Regression of atherosclerosis induced by liver-directed gene transfer of apolipoprotein A-I in mice. Circulation 1999; 100: 1816-22.

[159] Van Linthout S, Collen D, De Geest B. Effect of promoters and enhancers on expression, transgene DNA persistence, and hepatotoxicity after adenoviral gene transfer of human apolipoprotein A-I. Hum Gene Ther 2002; 13: 829-40.

[160] Zhang Y, Zanotti I, Reilly MP, Glick JM, Rothblat GH, Rader DJ. Overexpression of apolipoprotein A-I promotes reverse transport of cholesterol from macrophages to feces in vivo. Circulation 2003; 108: 661-3.

[161] Nanjee MN, Crouse JR, King JM, Hovorka R, Rees SE, Carson ER*, et al.* Effects of intravenous infusion of lipid-free apo A-I in humans. Arterioscler Thromb Vasc Biol 1996; 16: 1203-14.

[162] Nanjee MN, Doran JE, Lerch PG, Miller NE. Acute effects of intravenous infusion of ApoA1/phosphatidylcholine discs on plasma lipoproteins in humans. Arterioscler Thromb Vasc Biol 1999; 19: 979-89.

[163] Eriksson M, Carlson LA, Miettinen TA, Angelin B. Stimulation of fecal steroid excretion after infusion of recombinant proapolipoprotein A-I. Potential reverse cholesterol transport in humans. Circulation 1999; 100: 594-8.

[164] Nissen SE, Tsunoda T, Tuzcu EM, Schoenhagen P, Cooper CJ, Yasin M*, et al.* Effect of recombinant ApoA-I Milano on coronary atherosclerosis in patients with acute coronary syndromes: a randomized controlled trial. Jama 2003; 290: 2292-300.

[165] Gomaraschi M, Baldassarre D, Amato M, Eligini S, Conca P, Sirtori CR*, et al.* Normal vascular function despite low levels of high-density lipoprotein cholesterol in carriers of the apolipoprotein A-I(Milano) mutant. Circulation 2007; 116: 2165-72.

[166] Tardif JC, Gregoire J, L'Allier PL, Ibrahim R, Lesperance J, Heinonen TM*, et al.* Effects of reconstituted high-density lipoprotein infusions on coronary atherosclerosis: a randomized controlled trial. Jama 2007; 297: 1675-82.

[167] Navab M, Anantharamaiah GM, Hama S, Garber DW, Chaddha M, Hough G*, et al.* Oral administration of an Apo A-I mimetic Peptide synthesized from D-amino acids dramatically reduces atherosclerosis in mice independent of plasma cholesterol. Circulation 2002; 105: 290-2.

[168] Navab M, Shechter I, Anantharamaiah GM, Reddy ST, Van Lenten BJ, Fogelman AM. Structure and Function of HDL Mimetics. Arterioscler Thromb Vasc Biol 2009.